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We developed three iterative prototypes of a bioreactor system that facilitates microperfusion of tissue-like structures formed from primary liver cells, and showed that the bioreactor and detection system could be used in a field setting running on batteries. We demonstrated that the endogenous P450 metabolic capacity could be used as a sensor and that cells in the bioreactor were sensitive to toxins requiring liver metabolism

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FINAL REPORT

GRANT #: N00014-98-1-0760

PRINCIPAL INVESTIGATOR: Linda G. Griffith

INSTITUTION: Massachusetts Institute of Technology

GRANT TITLE: Vascularized Tissue Sensors for Generic Toxin and Pathogen Detection

AWARD PERIOD: 7/1/98 - 6/24/02

OBJECTIVE: To develope an *in vitro* tissue-based sensor based on primary liver cells, and demonstrate the performance of this sensor in detecting a set of toxin including aflatoxin B1, an agent which requires liver metabolism, using a novel optical readout based on cell metabolism.

APPROACH: Building on observations of histotypic 3D reorganization made by previous investigators as well as our own observations regarding reorganization of mixed hepatocyte-endothelial cells cultures, we developed a microfabricated bioreactor that allows us to recreate many features of three dimensional liver tissue at the level of the smallest functional tissue unit, the capillary bed. Further, the design of the reactor allows one to probe tissue structure and function in a noninvasive manner via microscopy. We exploited silicon microfabrication technology to create an array of capillary-bed size channels in a thin silicon chip to serve as a scaffold for 3D tissue morphogensis. Using a computational fluid dynamics model as a guide, we designed a flow-through reactor housing that allows cells placed in the silicon scaffold to be perfused with nutrient medium at flow rates that provide physiological shear stresses and nutrient delivery rates. The system can be scaled to hold 10^4 - 10^6 cells by adjusting the number of holes in the chip, each of which hold ~10^3 cells, and by appropriate scaling of the reactor housing. Further, the housing is designed with dimensions that allow the tissue to be within the focal plane of a light microscope or 2-photon microscope so that repeated in situ observation of tissue behavior is possible. Test agents can be added to the circulating medium

ACCOMPLISHMENTS

We developed an Integrated sensor that can run on battery power. The final system comprises

(1) a microfabricated bioreactor for differentiated tissue culture with protocols for operation with primary hepatocytes, co-cultures of hepatocytes and endothelial cells, and (co) cultures of liver epithelial cell lines; The reactor is a fourth generation bioreactor (MilliRxrF), designed using computational modeling of fluid flow and

mass transfer and incorporating user suggestions from experience with earlier versions.

(2) millifluidic system for distribution of culture medium and reagents with controllled temperature and gas phase humidity/CO_2. This system can be used to culture cells for a period of several days outside the incubator (e.g., room air/temperature).

(3) A miniaturized optical system enabling sequential detection of 3 different fluorescence readouts (EROD/P450; Sytox Nuclear stain; b-lactamase; plus GFP for development work) from one culture within a 10 min measurement period (filter switching required). Four units have been fabricated that are capable of detecting the desired readouts. Results from feasibility studies have led us to focus on P450 (resorufin) as the primary readout for sensor operation, and to GFP for characterization of viral infection.

Performance of the system was demonstrated using aflatoxin, microcystin, shiga toxin

Aflatoxin: Aflatoxin B1 is a natural product of molds that grow on grain, but it is also a potential biowarfare agent. It requires liver metabolism for toxicity, and has a complex metabolic pathway, requiring liver enzymes that are typically rapidly lost in 2D culture. Performance of the system to detect AFB1 toxicity was tested with P450 1A1 ethyoxyresorufin readouts. In this assay, the substrate ethoxyresorufin is converted to the fluorescent product resorufin by active intracellular processes, and cell toxicity is reflected by a decrease in signal compared to control. A limit of detection of 5 ng/ml within 24 hours with P450 readout; similar sensitivity was seen for albumin secretion suppression and LDH leakage. *An NIEHS grant was obtained to characterize the molecular basis of aflatoxin response in the bioreactor, focusing on transcriptional profiling to compare the bioreactor to the in vivo state in baseline and aflatoxin-treated conditions.*

Microcystin: Microcystin is a small peptide toxin produced by cyanobacteria as a natural product, and is found endemic in certain parts of the world in water where bacterial blooms are common. Because microcystin is resistant to filtration and chlorine, it is viewed as a potential biowarfare agent. The system was shown to detect 5 microM microcystin within an hour.

Shiga Toxin: Shiga toxin is a bacterial product that causes loss of blood vessel integrity through action on endothelia. A high degree of variability in setting up endothelial co-cultures limited progress on the Shiga toxin response. Initial co-cultures showed little response by live-dead assay. *Further work in assessing toxicity in co-cultures is continuing.*

3. **Feasibility of Virus detection:**

Adenovirus: Adenovirus was chosen as a model virus to characterize the distribution and uptake of a virus in the system. A non-replicating Ad5 vector was used to deliver the gene for eGFP under the CMV promoter, allowing non-invasive analysis of viral uptake and gene expression by measuring fluorescence using the spectrometer. The rate of viral uptake and expression depends on dose, as expected. *This work is being continued under NSF funding.*

CONCLUSIONS: We developed three iterative prototypes of a bioreactor system that facilitates micro-perfusion of tissue-like structures formed from primary liver cells, and showed that the bioreactor and detection system could be used in a field setting running on batteries. We demonstrated that the endogenous p450 metabolic capacity could be used as a sensor and that cells in the bioreactor were sensitive to toxins requiring liver metabolism.

SIGNIFICANCE: Because liver metabolic functions are labile in traditional cultures, the microperfused liver format is a promising advance in providing extended high-level function in culture. The system is being further developed as a means of testing drug toxicity and toxicity of environmental agents.

PATENT INFORMATION: .
Griffith, L.G., Tannenbaum, S.R., Powers, M.J., Thompson, C., Domansky, K., "Vascularized Micro Tissue/Micro Organ Arrays," Patent No. 6,197,575

AWARD INFORMATION:
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